Reference: *Biol. Bull.* **180**: 295–300. (April, 1991)

Control of Central and Peripheral Targets by a Multifunctional Peptidergic Interneuron

DAVID J. PRIOR

Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011

Abstract. In the terrestrial slug, *Limax maximus*, feeding activity and cardiovascular function have been shown to be correlated. For example, in intact animals, both feeding responsiveness and heart activity are suppressed during dehydration (Grega and Prior, 1986). The paired peptidergic buccal ganglion neurons RB1 and LB1 have dramatic modulatory effects on both the feeding motor program (FMP) and the force of heart contraction (Welsford and Prior, 1991). The B1 neurons appear to contain the small cardioactive peptides (SCPs). Observations have a frequency dependent excitation of both the FMP and the heart demonstrated by intracellular stimulation of B1. Thus, interneuron B1 may serve to mediate the coincident modulation of multiple responses to physiological stresses.

Introduction

Environmental stress or a change in the physiological state of an organism very often results in a concerted array of regulatory responses. Such responses usually include modification of behavioral patterns, or the level of behavioral responsiveness, as well as changes in physiological functions such as cardiac output and respiratory activity. With the use of certain invertebrate organisms, recent research has addressed the question of the control of such concerted response patterns (Prior, 1989; Teyke *et al.*, 1990; Frugal and Brownell, 1987).

Terrestrial gastropods, such as *Limax maximus*, are remarkably susceptible to environmental stresses such as dehydration. In a drying environment, they can lose 30–40% of their body weight within a few hours (see Prior *et al.*, 1983; Riddle, 1983; Prior, 1985, for reviews). Among the array of regulatory responses displayed by dehydrating slugs are contact-rehydration (Prior, 1984; Prior and Uglem, 1984), modifications in respiratory function (Dickinson *et al.*, 1988), alterations in feeding responsiveness (Prior, 1983; Phifer and Prior, 1985) and modifications in cardiovascular function (Grega and Prior, 1986; Welsford and Prior, 1991). As such, *Limax* represents a useful model for the analysis of the integration of multiple regulatory responses.

The concerted control of feeding behavior and cardiovascular function in *Limax* has been a focus of recent work (see Grega and Prior, 1985; Prior and Welsford, 1989). Rhythmic feeding behavior in this organism involves alternating protraction and retraction of the toothed radula against a food source. Feeding bouts often last many minutes and can involve hundreds of bite cycles (see Gelperin *et al.*, 1978). In semi-intact or isolated preparations of the central nervous system (CNS: Fig. 1), chemical stimuli applied to the lips or electrical stimulation of the lip nerves can elicit a prolonged pattern of efferent neural activity that underlies the feeding movements. This feeding motor program (FMP; Prior and Gelperin, 1977; Gelperin *et al.*, 1978) consists of alternating bursts of activity in protractor and retractor motoneurons (Fig. 1, 2). In addition to activation of the major buccal musculature, the FMP involves synchronized activation of the accessory salivary system. During feeding, the activity of the fast salivary burster neurons (FSBs), which are the motoneurons to the salivary ducts, becomes phase-locked with protraction (Fig. 2).

SCP_B Modulation of Feeding and Heart Function

In gastropods, the small cardioactive peptides (SCPs) have an excitatory effect upon both the musculature (see Lloyd and Willows, 1988; Lloyd, 1989) and the neural networks underlying patterned feeding activity (see Willows *et al.*, 1988). In several species, SCP_B can initiate patterned efferent activity in isolated CNS preparations (*e.g.*, *Helisoma*, Murphy *et al.*, 1985; *Tritonia*, Willows

Received 14 December 1990; accepted 30 January 1991.

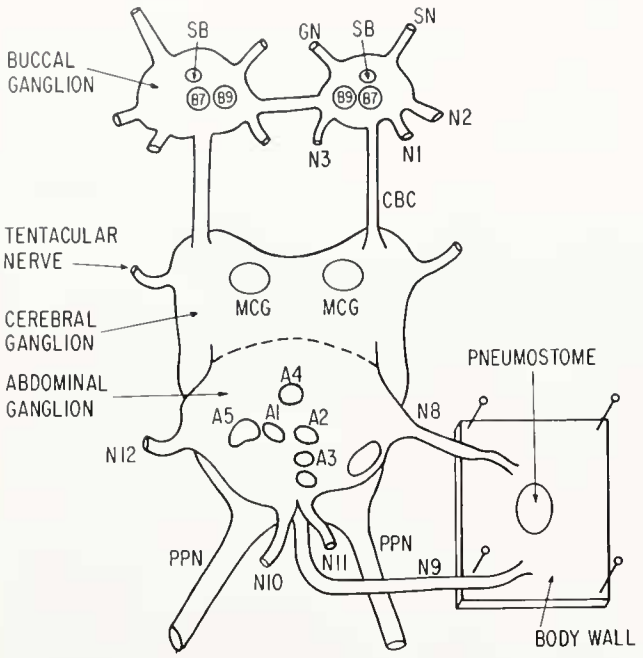

Figure 1. A diagram of the isolated central nervous system of *Limax* including: the paired buccal ganglia and the fused cerebral and abdominal ganglia; the pneumostome region; abdominal nerves N8–N12 and the posterior pedal nerves, PPN; buccal nerves N1–N3; gastric nerve, GN; salivary nerve, SN; buccal protractor motoneuron B7; fast salivary burster neuron, SB; cerebrobuccal connective, CBC; metacerebral giant cell, MCG.

et al., 1988). In *Limax*, however, SCP_B has a modulatory role, increasing the responsiveness of the central pattern generator to stimuli (Prior and Watson, 1987). In the presence of 10^{-7} to 10^{-6} *M* SCP_B, otherwise ineffective stimuli can initiate full expression of the feeding motor program.

Among the neurons in *Limax* that are responsive to SCP_B are the paired fast salivary bursters. The rate of endogenous burst activity in these motoneurons is enhanced by application of SCP_B in a concentration-dependent manner (Fig. 3, 4). Short-term application of SCP_B results in a slow increase in FSB burst frequency and an even slower decrement of the effect follows initiation of a saline wash. In addition, continuous perfusion of a preparation for 20–30 min reveals no indication of desensitization of the effect. In 10^{-6} *M* SCP_B, the burst frequency was sustained at 14 bursts/min compared with a control frequency of 1 burst/min (see Prior and Watson, 1987). It has been determined that this excitatory effect is mediated by an increase in the rate of the interburst depolarization rather than a general decrease in resting potential (Hess and Prior, 1989). Thus the effects of SCP_B on the *Limax* feeding system include modulation of the responsiveness of the FMP in addition to direct excitation of specific motoneurons.

To assess further the potential role of an SCP_B-like peptide in the regulation of feeding responsiveness, exogenous

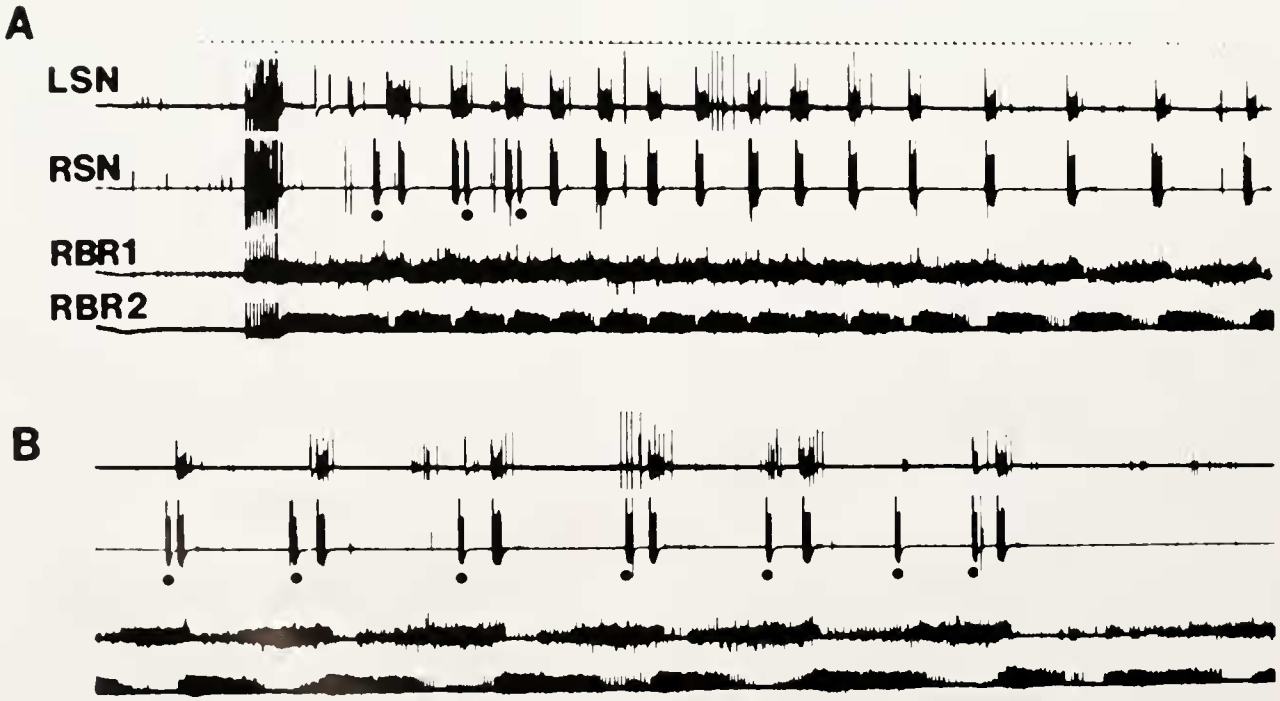

Figure 2. Activation of the feeding motor program (FMP) in an isolated buccal ganglia-brain preparation by electrical stimulation of an external lip nerve (artifacts at beginning at A). The FMP is characterized by alternation of efferent bursts correlated with protraction (buccal nerve 1: RBR1; and the right and left salivary nerves: RSN, LSN), and retraction (buccal nerve 2: RBR2). The nonfeeding endogenous bursts of the right FSB are noted with dots. The upper calibration trace indicates one mark/second. (From Prior and Watson, 1987)

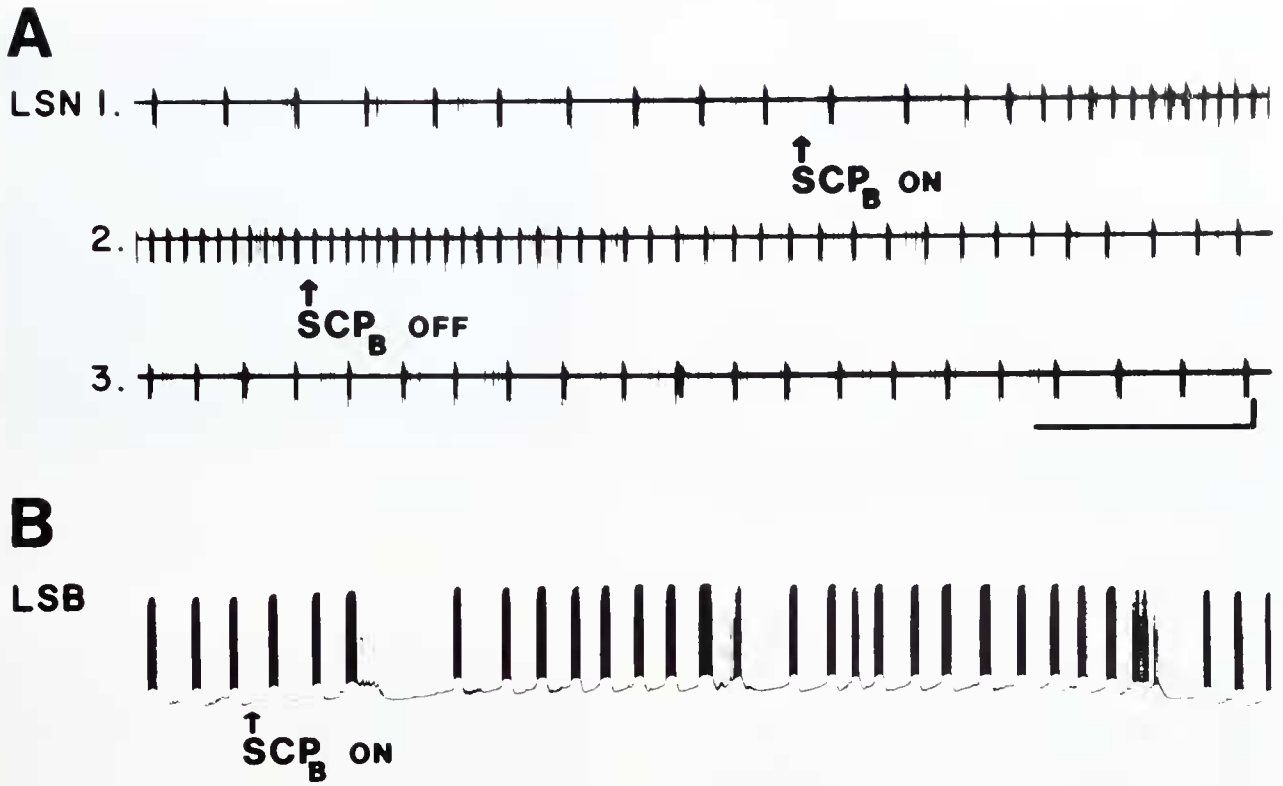

Figure 3. (A) A continuous extracellular recording from the left salivary nerve (LSN) of an isolated buccal ganglia-brain preparation is shown in 1–3. The prominent bursting unit in this record is the fast salivary burster (FSB; each burst consists of 12–15 spikes). Within 20 s of the application of 2×10^{-6} *M* SCP_B to the preparation (first arrow), the burst frequency of the FSB increases. Following removal of SCP_B from the superfusion medium (second arrow), burst frequency of the FSB returns to the pretreatment level. (B) an intracellular recording from the fast salivary burster neuron (FSB) showing the increase in burst frequency and, in this case, progressive depolarization, in response to 2×10^{-6} *M* SCP_B (the dashed line indicates the level of the interburst hyperpolarization before exposure to SCP_B). Bar = 30 s (A) and 20 mV (B). (From Prior and Watson, 1988)

SCP_B was injected into intact animals and their feeding responsiveness measured. As shown in Table I., SCP_B can initiate the apetetive phases of feeding behavior including: (1) cessation of locomotion, (2) tentacular retraction, (3) lip eversion, and (4) lip movement. That the consumatory phase of feeding was not regularly initiated was not unexpected, in that in isolated CNS preparations SCP_B did not initiate feeding, but rather, increased responsiveness to stimuli. Nevertheless, this would appear to be the first demonstration of an orderly effect of injected SCP_B in an intact organism. This result certainly supports the notion that an SCP_B-like peptidergic system is involved in the control of the feeding system in *Limax*.

The small cardioactive peptides have been shown to have an excitatory effect on the musculature of numerous systems, including *Helix* heart (Lloyd 1978, 1982), *Aplysia* and *Tritonia* buccal mass and gut (Lloyd *et al.*, 1984; Lloyd and Willows, 1988), and *Limax* ventricle (Welsford and Prior, 1991; Lloyd, 1979; 1989). In *Limax*, both SCP_B and SCP_A cause a concentration-dependent increase in the force of ventricular contraction (Welsford and Prior, 1991). At a concentration of 10^{-6} *M*, SCP_B can cause a 150% increase in the force of ventricular contractions. Although lower concentrations of SCP_B (10^{-9} to 10^{-7} *M*) can cause a slight increase in heart rate, there does not appear to be a consistent effect (Prior and Welsford, 1989).

The excitatory effects of SCP_B on heart and the feeding system of *Limax*, together with the stress-induced coincident changes in feeding and cardiovascular function observed in intact animals (Grega and Prior, 1985), are indicative of the possibility of coincident control of these two systems.

Multifunctional Modulatory Interneuron B1

In that exogenous SCP_B can simultaneously modify feeding and cardiovascular function, immunohistochemical techniques were used in an effort to identify central

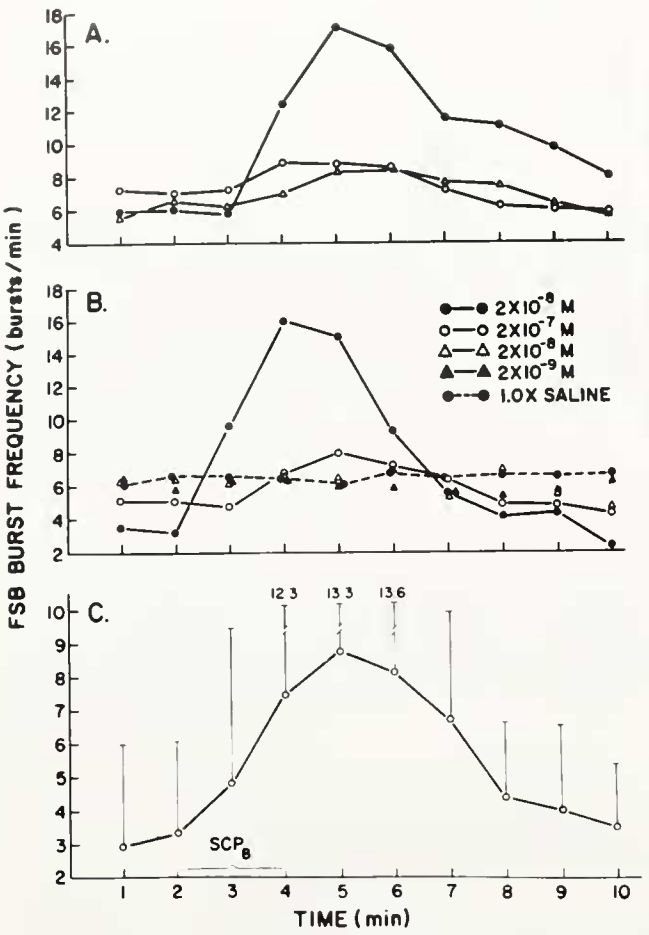

Figure 4. The responses of the fast salivary burster neuron (FSB) to varying concentrations of SCP_B are presented by plotting burst frequency as a function of time during the experiment. In each case, SCP_B was superfused over an isolated buccal ganglion-brain preparation between minutes 2 and 4. The preparation was superfused with saline for 20 min between each trial. (A) The responses obtained in three trials with the same preparation using various concentrations of SCP_B are shown. Each point represents the burst frequency of the FSB in the preceding 60 s. (B) The responses of a second preparation to SCP_B. In this case four different concentrations of SCP_B were used as well as a control saline trial. (C) The extent of the variability between preparations is illustrated by plotting the mean (±SD) burst frequency at each time point for 29 trials in 12 preparations during exposure to 2×10^{-6} *M* SCP_B. (From Prior and Watson, 1987)

neurons containing SCP_B-like-immunoreactive-material (SLIM) that might be involved. Among the most prominent SLIM-reactive neurons were the right and left B1 buccal neurons (Prior and Watson, 1987). In addition to those neurons that clearly contain SCP_B immunoreactive material, there are numerous cell bodies that are enmeshed by networks of immunoreactive fibers (*e.g.*, B7, FSB), which is suggestive of peptidergic endings near the target feeding neurons.

The morphology of B1 was examined by intracellular injection of horseradish peroxidase (Fig. 5a). There are

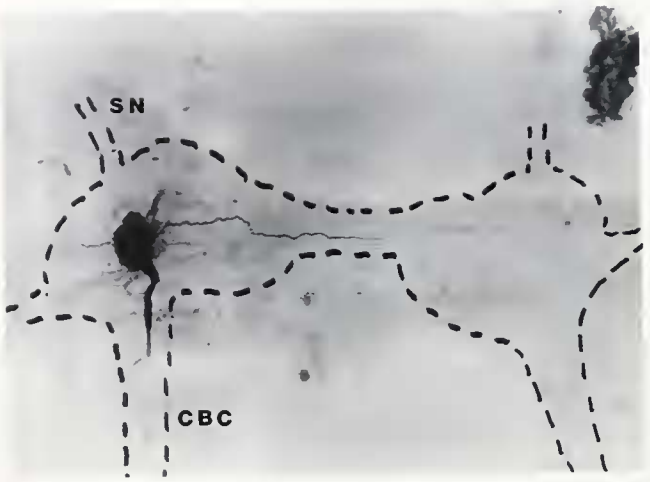

Figure 5a. A photomicrograph of a preparation of paired buccal ganglia (outlined) showing the morphology of the left B1 neuron injected with horseradish peroxidase. Cerebrobuccal connective, CBC; salivary nerve, SN; this preparation was made by K. Delaney)

two major axonal projections and an extensive dendritic arborization in the lateral lobe of the buccal ganglion. A small axon projects across the buccal-buccal commissure.

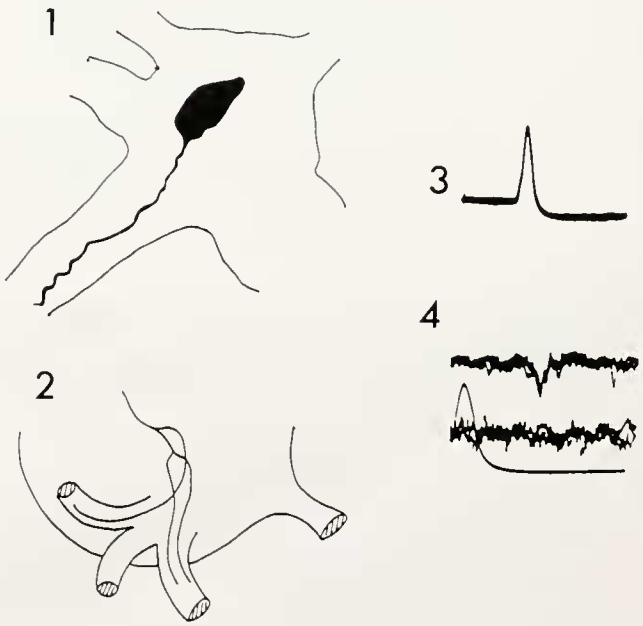

Figure 5b. Panel 1 is a camera lucida drawing of the soma of LB1 following injection of Co^{++} showing the major axon exiting the buccal ganglion in the ipsilateral cerebrobuccal connective. Panel 2 is a camera lucida drawing of the abdominal ganglion showing the continued axonal projection of the injected LB1, with one axonal branch in abdominal ganglion nerve 9 and two axonal branches in nerve 11. Panel 3 illustrates antidromic activation of B1 in response to repetitive stimulation of the cardiac branch of nerve 9. Panel 4 shows repetitive intracellular stimulation of B1 causing in a constant-latency axonal impulse recorded in nerve 9.

Table I

Behavioral effects of SCP_B injections in Limax maximus

Treatment	Behavioral observations			
	Tentacular locomotion	Lip retraction	Lip eversion	Lip movement
Saline	83.0%	8.0%	0.0%	0.0%
10^{-7} moll^{-1} SCP_B	83.0%	25.0%	17.0%	8.0%
10^{-6} moll^{-1} SCP_B*	92.0%	33.0%	42.0%	25.0%
10^{-5} moll^{-1} SCP_B**	17.0%	83.0%	100%	58.0%

The percentage of animals that displayed each behavior is presented in each case.

Each animal received injections of each concentration of SCP_B and the saline control.

The 0.05 probability level was accepted as significant (determined by a Friedman's test and a non-parametric multiple comparisons procedure).

All concentrations of SCP_B are the calculated final hemolymph concentrations.

The results with 1^{-5} and 10^{-6} moll^{-1} SCP_B injection were significantly different from those with injection of control saline and 10^{-7} moll^{-1} SCP_B. Furthermore, the results with 10^{-5} moll^{-1} SCP_B injection were significantly greater than those observed with injection of 10^{-6} moll^{-1} SCP_B.

* $P < 0.05$, ** $P < 0.01$, n = 12.

(From Schagene *et al.*, 1989)

Although the dendritic arborizations occur primarily in the lateral lobe, they do span into the medial lobe, including the region containing both retractor and protractor feeding motoneurons. The major axon projects out the ipsilateral cerebrocuccal connective, through the cerebral ganglion, into the abdominal ganglion and out abdominal nerves 9 and 11, which innervate the heart and kidney complex, respectively (Fig. 5b). Rapid stimulation (5Hz) of a cardiac branch of nerve 9 resulted in antidromic activation of the soma of B1. Correspondingly, repetitive activation of the soma of B1 by intracellular current injection was followed by a constant latency impulse in the cardiac branch of nerve 9 (Fig. 5a).

The immunohistochemical results together with the basic morphology of B1, including significant arborizations in the region of the feeding neurons, and, remarkably, a major axonal projection to the heart, were suggestive of a role for B1 in the concerted control of feeding and cardiovascular functions.

Intracellular stimulation of B1 at quite low frequencies results in a progressive increase in the activity of the fast

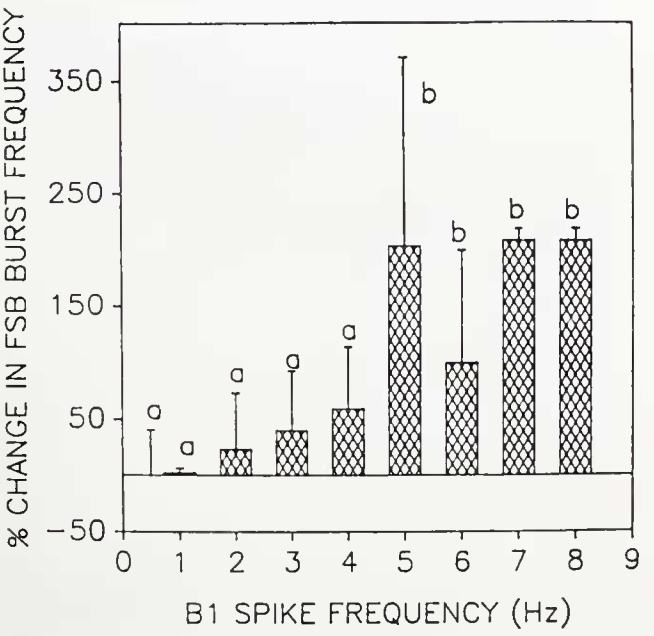

Figure 6. A summary of the change in the burst frequency of the fast salivary burster neuron initiated by intracellular stimulation of the ipsilateral B1 neuron at different impulse frequencies. The apparent threshold frequency for B1 is 2–4 Hz with the maximal effect occurring at about 7 Hz. Changes in the FSB burst frequency were normalized as a percentage of the pre-stimulation level of activity in each preparation. Each bar represents the mean (±SD) response of five buccal ganglion-brain preparations. (From Prior and Welsford, 1989)

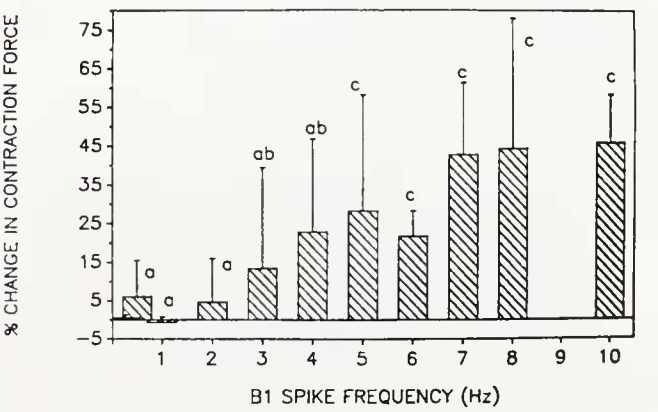

Figure 7. The effect of unilateral stimulation of B1 on the force of ventricular contraction. The bars represent the mean (±SD) response from 56 trials. Ten action potentials were elicited at each frequency. The a, b, c notation refers to significant differences (*e.g.*, C's are significantly, $P < 0.001$, different from a's and b's). (Redrawn from data of Welsford and Prior, 1991)

salivary bursters. As shown in Figure 6, driving B1 at 5 impulses/s can result in a 50% increase in FSB burst frequency. Even two to three impulses at low frequencies are sufficient to elicit a transient increase in FSB burst frequency. These effects are sustained in high Mg^{++}, high Ca^{++} saline indicating the possibility of monosynaptic connection.

To assess the potential role of B1 in the control of heart function, semi-intact preparations of the CNS and innervated heart were used, which allowed intracellular stimulation of B1 and measurement of ventricular activity. Stimulation of B1 at low frequencies resulted in an increase in the force of contraction of the heart (Fig. 7). It is of interest that B1 frequencies of 5 to 7 impulse/s were the most effective in the activation of both the FSB and the heart.

When this experiment was repeated with the CNS bathed in high Mg^{++}, high Ca^{++} saline, there was no change in the effectiveness of B1 to increase heart function. This suggests that B1 has a direct effect on peripheral targets rather than acting via additional CNS neuronal pathways.

Thus, it would appear that buccal neuron B1 may be a multifunction peptidergic interneuron capable of simultaneously modulating the central feeding motor program and cardiovascular function. As such, B1, along with other similar neurons, is positioned to control the synchrony of multiple behavioral responses normally observed in response to environmental stress and changes in the physiological state of an organism.

Acknowledgments

The work described in this paper was supported, in part, by grants from the Arizona Disease Control Research Commission (#82-0698) and The National Institutes of Health (M.B.R.S.# 2 SO3 RR03401-03).

Literature Cited

Dickinson, P. S., D. J. Prior, and C. Avery. 1988. The pneumostome closure rhythm in slugs: a response to dehydration controlled by hemolymph osmolality and peptide hormones. *Comp. Biochem. Physiol.* **89A**(4): 579–586.

Furgal, S. M., and P. H. Brownell. 1987. Ganglionic circulation and its effects on neurons controlling cardiovascular functions in the *Aplysia californica*. *J. Exp. Zool.* **244**: 347–364.

Gelperin, A., J. J. Chang, and S. Reingold. 1978. Feeding motor program in *Limax maximus:* neuromuscular correlates and control by chemosensory input. *J. Neurobiol.* **9**: 285–300.

Grega, D. S., and D. J. Prior. 1985. The effects of feeding on heart activity in the terrestrial slug, *Limax maximus:* central and peripheral control. *J. Comp. Physiol.* **156A**: 539–545.

Grega, D. S., and D. J. Prior. 1986. Modification of heart activity in response to dehydration in the terrestrial slug. *Limax maximus*. *J. Exp. Biol.* **237**: 185–190.

Hess, S. D., and D. J. Prior. 1989. Small cardioactive peptide B modulates feeding motoneurons in *Limax maximus*. *J. Exp. Biol.* **142**: 473–478.

Lloyd, P. E. 1978. Distribution and molecular characteristics of cardioactive peptides in the snail, *Helix aspersa*. *J. Comp. Physiol.* **128**: 269–276.

Lloyd, P. E. 1979. Central peptide containing neurons modulate gut activity in *Tritonia*. *Soc. Neurosci. Abstr.* **5**: 252.

Lloyd, P. E. 1982. Cardioactive neuropeptides in gastropods. *Fed. Proc.* **41**: 2948–2852.

Lloyd, P. E. 1989. Peripheral actions of the SCPs in *Aplysia* and other gastropod molluscs. *Am. Zool.* **29**: 1265–1274.

Lloyd, P. E., I. Kupfermann, and K. R. Weiss. 1984. Evidence for parallel actions of a molluscan neuropeptide (SCP_A) and serotonin in mediating arousal in *Aplysia*. *Proc. Nat. Acad. Sci. USA* **81**: 2934–2937.

Lloyd, P. E., and A. O. D. Willows. 1988. Multiple transmitter neurons in *Tritonia* II. Control of gut motility. *J. Neurobiol.* **19**(1): 55–67.

Murphy, A. D., K. Lukowiak, and W. K. Stell. 1985. Peptide modulation of patterned motor activity in identified neurons of *Helisoma*. *Proc. Nat. Acad. Sci. USA* **82**: 7140–7144.

Phifer, C. B., and D. J. Prior. 1985. Body hydration and haemolymph osmolality affect feeding and its neural correlate in the terrestrial gastropod, *Limax maximus*. *J. Exp. Biol.* **118**: 405–426.

Prior, D. J. 1983. Hydration-induced modulation of feeding responsiveness in terrestrial slugs. *J. Exp. Zool.* **227**: 15–22.

Prior, D. J. 1984. Analysis of contact-rehydration in terrestrial gastropods: osmotic control of drinking behaviour. *J. Exp. Biol.* **111**: 63–73.

Prior, D. J. 1985. Water-regulatory behaviour in terrestrial gastropods. *Biol. Rev.* **60**: 403–424.

Prior, D. J., and G. L. Uglem. 1984. Analysis of contact-rehydration in terrestrial gastropods: absorption of ^{14}C-inulin through the epithelium of the foot. *J. Exp. Biol.* **111**: 73–80.

Prior, D. J. 1989. Neuronal control of osmoregulatory responses in gastropods. Pp. 1–24 in *Advances in Comparative Environmental Physiology*, R. Gilles, ed. 5. Springer-Verlag, New York.

Prior, D. J., and A. Gelperin. 1977. Autoactive molluscan neuron: reflex function and synaptic modulation during feeding in the terrestrial slug, *Limax maximus*. *J. Comp. Physiol.* **114**: 217–232.

Prior, D. J., and W. H. Watson. 1987. The molluscan neuropeptide, SCP_B, increases the responsiveness of the feeding motor program of *Limax maximus*. *J. Neurobiol.* **19**(1): 87–105.

Prior, D. J., and I. G. Welsford. 1989. The role of small cardioactive peptide, SCP_B, in the regulatory responses of terrestrial slugs. *Am. Zool.* **29**: 1255–1263.

Prior, D. J., M. Hume, D. Varga, and S. D. Hess. 1983. Physiological and behavioural aspects of water balance and respiratory function in the terrestrial slug, *Limax maximus*. *J. Exp. Biol.* **104**: 111–127.

Riddle, W. A. 1983. Physiological ecology of land snails and slugs. Pp. 431–461 in *The Mollusca*, W. D. Russell-Hunter, ed. *Ecology*, 6. Academic Press, New York.

Schagene, K. A., I. G. Welsford, D. J. Prior, and P. A. Banta. 1989. Behavioral effects of injection of small cardioactive peptide, SCP_B, on the slug *Limax maximus*. *J. Exp. Biol.* **143**: 553–557.

Teyke, T., K. R. Weiss, and I. Kupfermann. 1990. An identified neuron (CPR) evokes neuronal responses reflecting food arousal in *Aplysia*. *Science* **247**: 85–87.

Welsford, I. G., and D. J. Prior. 1991. Modulation of heart activity in the terrestrial slug *Limax maximus* by the feeding motor program, small cardioactive peptides and stimulation of buccal neurone B1. *J. Exp. Biol.* **155**: 1–19.

Willows, A. O. D., P. E. Lloyd, and B. P. Masinovsky. 1988. Multiple transmitter neurons in *Tritonia* III. Modulation of central pattern generator controlling pattern. *J. Neurobiol.* **19**(1): 69–86.